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Published:
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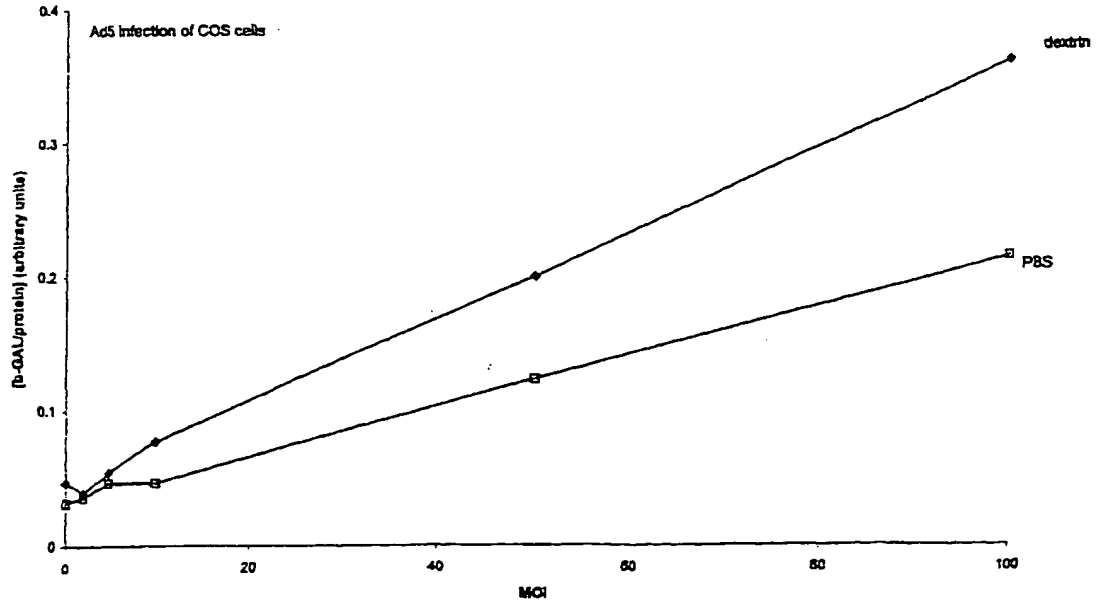
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR DELIVERY OF THERAPEUTIC AGENTS USING A SOLUTION OF DEXTRIN



(57) Abstract: The invention herein described relates to the delivery of therapeutic agents and in particular genetic material, to an animal in combination with dextrin.

WO 01/12231 A1

solution to fall. Because of this, the initial osmolality of the solution must be made fairly high (by using a sufficiently high concentration of dextrose) in order that the solution continues to effect dialysis for a reasonable length of time before it has to be withdrawn and replaced by fresh solution.

Other osmotic agents have been proposed for use in peritoneal dialysis and in recent years dextrin (a starch hydrolysate polymer of glucose) has been used. When instilled in the peritoneal cavity, dextrin is slowly absorbed via the lymphatic system, eventually reaching the peripheral circulation. The structure of dextrin is such that amylases break the molecule down into oligosaccharides in the circulation. These are cleared by further metabolism into glucose.

Dextrin solutions have been proposed as the medium for delivery of drugs to the body via the peritoneum. In GB-A-2207050, such a solution is proposed for the intraperitoneal administration of drugs for which enteral administration is unsatisfactory. Such an approach is stated to be particularly useful for the delivery of peptide drugs such as erythropoetin and growth hormones. Reference is also made to cephalosporin antibiotics. The concentration of dextrin in the aqueous solution is stated to be preferably from 0.5 to 10% w/v and an example of a composition for the delivery of erythropoetin has a dextrin concentration of about 10% w/v.

Gene therapy is concerned, inter alia, with the transfer of genetic material to specific target cells of a patient to prevent or alter a particular disease state. The treatment involves the use of carriers or delivery vehicles, often termed vectors, adapted for the delivery of therapeutic genetic material. These vectors are usually viral but non-viral vectors are also known. Immunogene therapy involves the use of genes for immunotherapy, including the provision of gene-based vaccines.

The mesothelial lining of the peritoneal cavity comprises a lining of cells that cover a broad surface. The peritoneal mesothelium has good lymphatic drainage and permits

Promoter is an art recognised term and, for the sake of clarity, includes the following features which are provided by example only, and not by way of limitation. Enhancer elements are cis acting nucleic acid sequences often found 5' to the transcription initiation site of a gene (enhancers can also be found 3' to a gene sequence or even located in intronic sequences). Enhancers function to increase the rate of transcription of the gene to which the enhancer is linked. Enhancer activity is responsive to trans acting transcription factors (polypeptides) which have been shown to bind specifically to enhancer elements. The binding/activity of transcription factors (please see Eukaryotic Transcription Factors, by David S Latchman, Academic Press Ltd, San Diego) is responsive to a number of physiological/environmental cues which include, by example and not by way of limitation, intermediary metabolites (eg glucose, lipids), environmental effectors (eg light, heat,).

Promoter elements also include so called TATA box and RNA polymerase initiation selection (RIS) sequences which function to select a site of transcription initiation. These sequences also bind polypeptides which function, *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers and autonomous replication sequences which facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic host. Vectors which are maintained autonomously are referred to as episomal vectors. Episomal vectors are desirable since these molecules can incorporate large DNA fragments (30-50kb DNA). Episomal vectors of this type are described in WO98/07876.

Adaptations which facilitate the expression of vector encoded genes include the provision of transcription termination/polyadenylation sequences. This also includes the provision of internal ribosome entry sites (IRES) which function to maximise expression of vector encoded genes arranged in bicistronic or multi-cistronic expression cassettes. Expression control sequences also include so-called Locus

As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and thereby, inhibits the transcription of that gene and/or the translation of that mRNA. Antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence.

It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions.

In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 7 (Wagner et al., Nature Biotechnology 14:840-844, 1996) and more preferably, at least 15 consecutive bases which are complementary to the target. Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases.

Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. The 3'- untranslated regions are known to contain *cis* acting sequences which act as binding sites for proteins involved in stabilising mRNA molecules. These *cis* acting sites often form hair-loop structures which function to bind said

may include sugars such as arabinose instead of ribose. Modified oligonucleotides also can include base analogs such as C-5 propyne modified bases (Wagner et al., Nature Biotechnology 14:840-844, 1996).

The present invention, thus, contemplates pharmaceutical preparations containing natural and/or modified antisense molecules that are complementary to and hybridizable with, under physiological conditions, nucleic acids encoding proteins the regulation of results in beneficial therapeutic effects, together with pharmaceutically acceptable carriers (eg polymers, liposomes/cationic lipids).

Antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art (eg liposomes). The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism.

In addition gene therapy vectors and/or antisense oligonucleotides are typically combined with carriers, for example polymers, cationic lipids/liposomes.

The use of cationic lipids (eg liposomes, Felgner (1987) Proc.Natl.Acad.Sci USA, 84:p7413) has become a common method to introduce DNA into cells. The cationic head of the lipid associates with the negatively charged nucleic acid backbone of the DNA to be introduced. The lipid/DNA complex associates with the cell membrane and fuses with the cell to introduce the associated DNA into the cell. Liposome mediated DNA transfer has several advantages over existing methods. For example, cells which are recalcitrant to traditional chemical methods are more easily transfected using liposome mediated transfer.

Any dextrin is a mixture of polyglucose molecules of different chain lengths. As a result, no single number can adequately characterise the molecular weight of such a polymer. Accordingly various averages are used, the most common being the weight average molecular weight (Mw) and the number average molecular weight (Mn). Mw is particularly sensitive to changes in the high molecular weights content of the polymer whilst Mn is largely influenced by changes in the low molecular weight of the polymer.

It is preferred that the Mw of the dextrin is in the range from 1,000 to 200,000, more preferably from 2,000 to 55,000.

The term "degree of polymerisation" (DP) can also be used in connection with polymer mixtures. For a single polymer molecule, DP means the number of polymer units. For a mixture of molecules of different DP's, weight average DP and number average DP correspond to Mw and Mn. In addition DP can also be used to characterise a polymer by referring to the polymer mixture having a certain percentage of polymers of DP greater than a particular number or less than a particular number.

It is preferred that, in the present invention, the dextrin contains more than 15% of polymers of DP greater than 12 and, more preferably, more than 50% of polymers of DP greater than 12.

Preferably the dextrin is present in the solution in an amount of less than 10%.

Preferably the dextrin is present in the solution in an amount selected from: 1% (w/v);2%(w/v); 3%(w/v); 4%(w/v); 5%(w/v); 6%(w/v); 7%(w/v); 8%(w/v);9%(w/v); 10%(w/v).

More preferably the dextrin is present from 2 to 5% by weight, most preferably about 4% by weight.

Materials and Methods

Two reporter constructs were used to monitor the effect of dextrin on transfection effciency. Green Fluroescent Protein (GFP) reporter gene was used in an adeno-associated virus (AAV) vector located in an icodextrin solution. Alternatively, LacZ reporter was used to monitor transfection efficiency.

Transgene expression in normal cells in the peritoneal wall was demonstrated at vector concentrations of from 1 x 10^8 to 1 x 10^{10} PN/ml.

EXAMPLE 1

(i) Transfection of tissue culture cells with rAAV encoding a Green Fluorescent Protein (GFP) Reporter Gene.

80% confluent BHK cells in 10cm tissue culture dishes were transfected with a total of 30 µg plasmid DNA per plate using Lipofectin/Peptide 6/DNA complexes. The ratio of rAAV vector plasmid (encoding GFP) to packaging plasmid (encoding necessary replication and packaging signals) was 1:3.

(ii) Infection with Helpervirus

5 hours post transfection cells were infected at a multiplicity of infection (MOI) of 3 with a herpes helpervirus in complete medium.

(iii) Harvesting

Approximately 42 hrs after infection cells were harvested by scraping, pelleted by spinning at 3500rpm for 10 min and resuspended in 10ml of buffer (140mM NaCl, 5mM KCl, 0.7mM K_2HPO_4, 25mM TrisHCl-pH 7.4). The solution was freeze thawed four times between a dry ice/ethanol bath and a 37°C waterbath to lyze the

Example 1 results

i) Storage at 4°C/37°C

a) 25 µl samples (n=1) were thawed out each day and stored at 4°C and 37°C respectively. After 7 days samples were titred together with an aliquot not exposed to these temperatures (day 0 sample).

b) The 37°C experiment was repeated and samples (n=3) for both icodextrin and saline were stored for 96 hours and 40 hours. They were titred together with aliquots not exposed to this temperature.

ii) Repeated freeze-thawing

One aliquot of rAAV/dextrin and rAAV/saline was freeze-thawed repeatedly between dry-ice and 37°C waterbath and 25 µl samples (n=3) were taken after 0, 10 and 20 freeze-thawing cycles. Samples were then titred.

Titration

1) HeLa cells were seeded in 96well dishes (2 x 10^4 cells/well) prior to titration experiments to ensure cells were subconfluent.

2) Using 10 µl of each aliquot, tenfold serial dilutions were prepared in complete media in a total volume of 1ml;

-10 µl of aliquot plus 990 µl of medium gave a 1:100 dilution,

-100 µl of this 10^{-2} dilution was transferred to a second tube containing 900 µl of media, giving a 10^{-3} dilution,

-100 µl of this 10^{-3} was transferred to a third tube, etc.

3) 50 µl of each dilution was transferred to a second set of 1.5ml tubes and 2 µl of wt Ad (stock $5x10^9$pfu/ml) added before mixing.

4) Media was taken from the cells and rAAV/wtAd mixture was added to the cells.

5) Green cells were counted after 24 hours using an inverted fluorescence microscope.

6) The titre was calculated as follows:

30 green cells/50 µl in 10^{-6} dilution

Example 2 results

Referring to Figure 4a. 24 hours after addition of the viral vector the cytopathic effects of the virus are more marked in the absence of dextrin. Figure 4b shows that in the presence of 4% dextrin there is an increase in the amount of β-galactosidase produced compared to PBS control.

9. A method according to any of Claims 1-8 wherein said therapeutic agent is combined with at least one carrier and/or excipient.

10. A method according to Claim 9 wherein said carrier and/or excipient is liposome based.

11. A method according to any of Claims 1-10 wherein said dextrin comprises glucose molecules linked theretogether by equal to or less than 10% α 1-6 linkages.

12. A method according to any of Claims 1-10 wherein said dextrin comprises glucose molecules linked theretogether by equal to or less than 5% α 1-6 linkages.

13. A method according to any of Claims 1-12 wherein the molecular weight of dextrin is in the range 1000-200,000.

14. A method according to any of Claims 1-12 wherein said molecular weight of dextrin is in the range 2000-55,000.

15. A method according to any of Claims 1-14 wherein said dextrin solution consists of at least 15% of polymers with a degree of polymerisation equal to or greater than 12.

16. A method according to any of Claims 1-14 wherein said dextrin solution consists of at least 50% of polymers with a degree of polymerisation equal to or greater than 12.

17. A method according to any of Claims 1-16 wherein said dextrin solution is at least 10% (w/v) dextrin.

18. A method according to any of Claims 1-16 wherein said dextrin solution is at least 5% (w/v) dextrin.

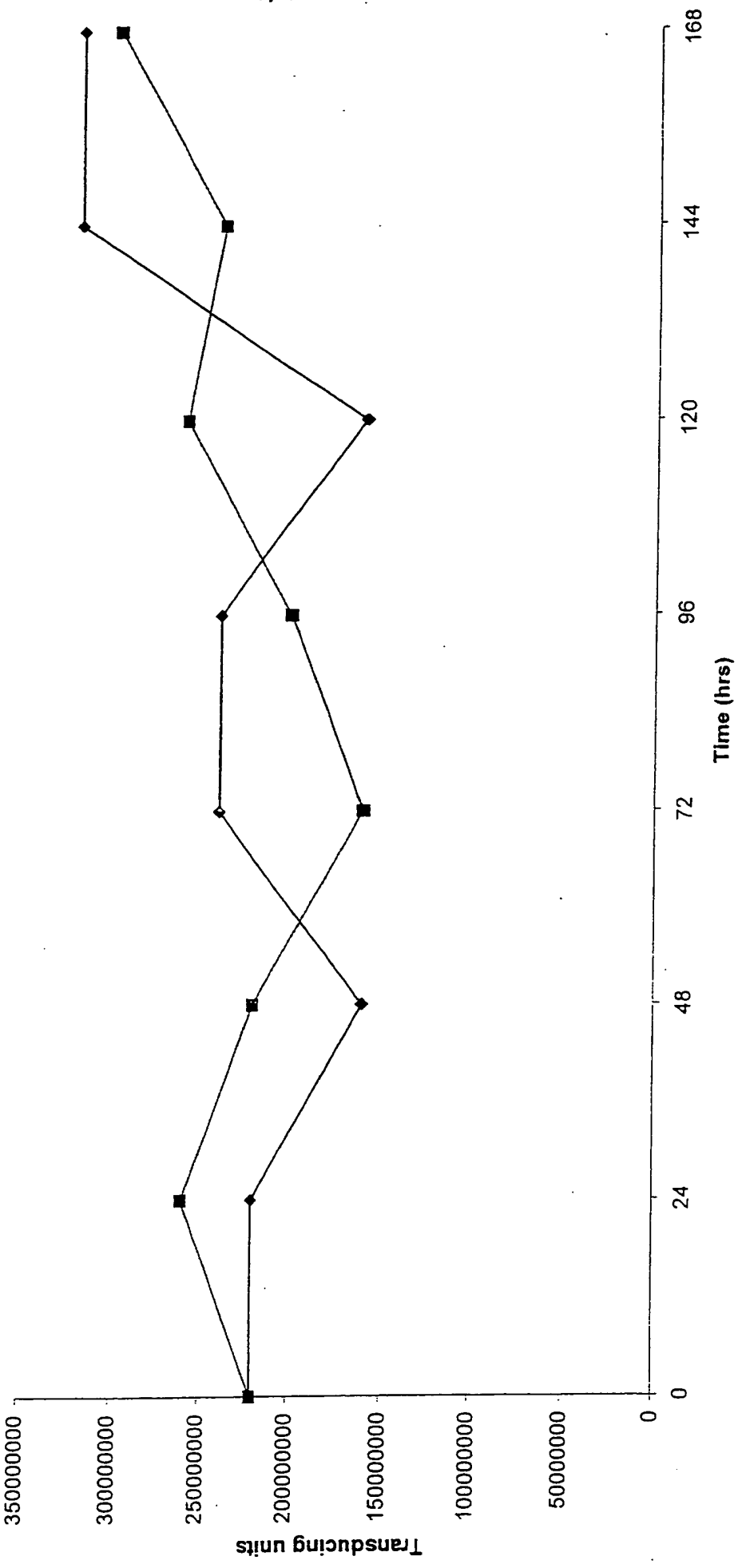

Transducing units
350000000
300000000
250000000
200000000
150000000
100000000
50000000
0
0
24
48
72
96
120
144
168
Time (hrs)

Figure 1

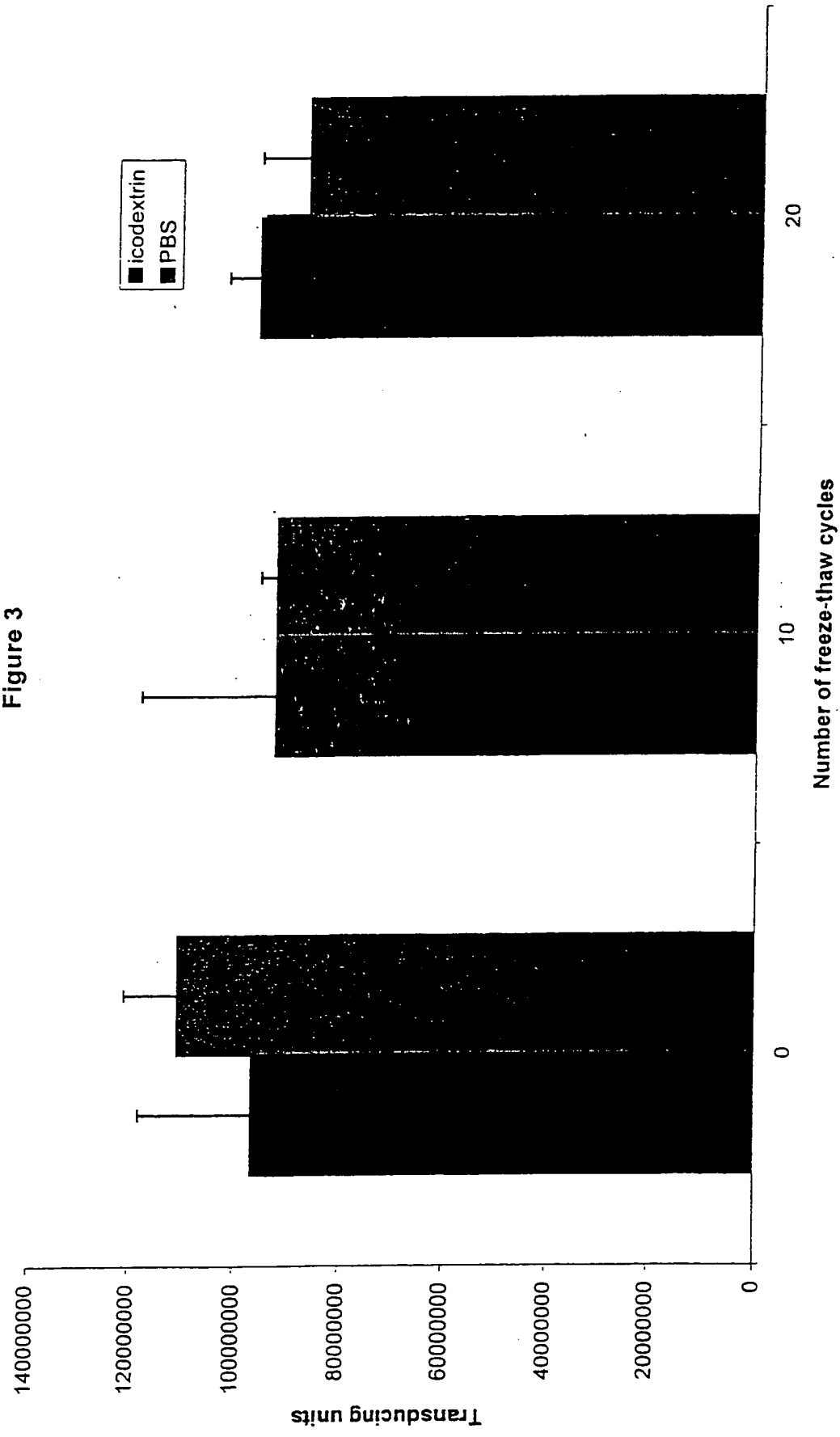

icodextrin
PBS
Transducing units
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
0
10
20
Number of freeze-thaw cycles

Figure 3

INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
PCT/GB 00/03025

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36 A61K48/00 C12N15/86 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 34325 A (ADVANCED MAGNETICS INC) 21 December 1995 (1995-12-21) the whole document, in particular page 17 line 25 to page 8 line 3 and claims 30, 33	1-10,20, 22
X	GB 2 207 050 A (HUNCHPLAN LIMITED) 25 January 1989 (1989-01-25) cited in the application	1,11-21
Y	the whole document, in particular page 10 last paragraph	2-10,22

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2000	12.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Julia, P

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/03025

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.